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Variants of the 3′ Region of the *cagA* Gene in *Helicobacter pylori* Isolates from Patients with Different *H. pylori*-Associated Diseases

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The CagA protein of *Helicobacter pylori* is an immunogenic antigen of variable size and unknown function that has been associated with increased virulence as well as two mutually exclusive diseases, duodenal ulcer and gastric carcinoma. The 3′ region of the *cagA* gene contains repeated sequences. To determine whether there are structural changes in the 3′ region of *cagA* that predict outcome of *H. pylori* infection, we examined 155 *cagA* gene-positive *H. pylori* isolates from Japanese patients including 50 patients with simple gastritis, 40 with gastric ulcer, 35 with duodenal ulcer, and 30 with gastric cancer. The 3′ region of the *cagA* gene was amplified by PCR followed by sequencing. CagA proteins were detected by immunoblotting using a polyclonal antibody against recombinant CagA. One hundred forty-five strains yielded PCR products of 642 to 651 bp; 10 strains had products of 756 to 813 bp. The sequence of the 3′ region of the *cagA* gene in Japan differs markedly from the primary sequence of *cagA* genes from Western isolates. Sequence analysis of the PCR products showed four types of primary gene structure (designated types A, B, C, and D) depending on the type and number of repeats. Six of the seven type C strains were found in patients with gastric cancer ($P < 0.01$ in comparison to noncancer patients). Comparison of type A and type C strains from patients with gastric cancer showed that type C was associated with higher levels of CagA antibody and more severe degrees of atrophy. Differences in *cagA* genotype may be useful for molecular epidemiology and may provide a marker for differences in virulence among *cagA*-positive *H. pylori* strains.

Helicobacter pylori is now recognized as the major causal agent of chronic gastritis, an indolent process, and although the pathogen is present in almost all patients, only a small proportion develop symptomatic disease such as peptic ulcer, gastric carcinoma, or primary gastric mucosa-associated lymphoid tissue lymphoma (6, 8, 21).

The experience with other bacterial pathogens suggests that *H. pylori* strain-specific factors may influence the pathogenicities of different *H. pylori* isolates (22). *H. pylori* strains have been divided into types I and II. Type I strains express CagA and vacuolating cytotoxin, VacA, whereas type II strains do not (2, 24). It has been suggested that duodenal-ulcer patients are more likely to be infected with type I strains (23, 24). Because inactivation of the *cagA* gene had no effect on the expression of VacA or on the ability to induce interleukin 8, it has been suggested that CagA is only a marker for increased virulence (4, 15, 20). The *cagA* gene is part of the *cag* pathogenicity island, a 40-kb DNA region, containing open reading frames that code for a putative *H. pylori* secretion system that may be associated with export of virulence factors to the extracellular compartment (2).

Several studies have reported an increased prevalence of CagA-positive *H. pylori* in gastric cancer (1, 14). However, studies done in Japan, Korea, and China have shown that more than 90% of *H. pylori* strains are *cagA* positive irrespective of clinical presentation (10, 12, 13, 16, 25). Recently, models have been proposed that would integrate the *H. pylori* virulence factors and environmental and host factors to possibly explain how the seemingly contradictory results might have developed (7).

The *cagA* gene product is a highly immunogenic outer membrane protein with a molecular weight of 120,000 (120K) to 140K. The *cagA* gene consists of an open reading frame encoding 1,147 to 1,181 amino acids (3, 19). The structure of the gene reveals a 5′ highly conserved region. Variation in the size of the protein has been correlated with the presence of a variable number of repeat sequences located in the 3′ region of the gene (3, 11).

The biological importance of the repeat regions in the 3′ end of the *cagA* gene is not known. Because CagA is strongly immunogenic, it is possible that the repeats affect the host immune response. For example, the repeats could be used to escape immunity by generating antigenic diversity or immunodominant nonprotective epitopes (3). The fact that there might be major differences in the 3′ region of the *cagA* gene was suggested by studies using strains from Korea. *H. pylori* strains from Korea were not detectable by PCR using primers that included the repeat sequences from Western isolates (12).

The present study was undertaken to study the variable regions of the 3′ region of *cagA* to identify whether differences in this region would be present in *H. pylori* isolates from patients with different *H. pylori*-related diseases in Japan, where the incidence of gastric carcinoma is among the highest in the world (235,000 cases, or 0.4% of the population, in 1993) (9).

MATERIALS AND METHODS

Isolates. The initial study population consisted of 491 patients who underwent gastric endoscopy with biopsy specimens for culture of *H. pylori* between 1995 and 1997 in the Third Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan. The *cagA* statuses of the 491 *H. pylori* isolates were assessed by PCR, and 476 (96.9%) strains were *cagA* positive (26). We selected 155 Japanese patients infected with *cagA*-positive strains, sex

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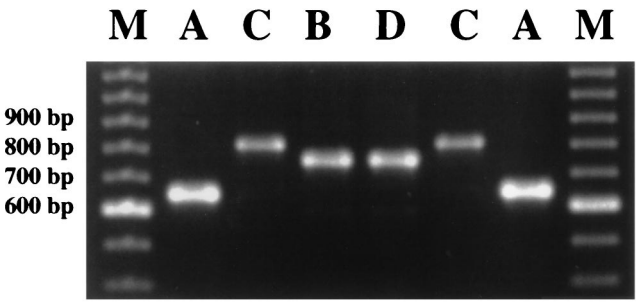

FIG. 1. Analysis of the 3′ region of the *cagA* gene by PCR. PCR products from a representative group of strains are shown. The sizes of the DNA fragments were confirmed after sequencing of the PCR products. Fragments A had sizes ranging from 642 to 651 bp. Sequencing showed that the primary structure of the *cagA* gene of two strains with 756-bp PCR fragments was different, and these strains were designated B and D. Fragments C were 810-bp products. Lanes M, molecular size markers.

matched and age matched among different diseases (78 men and 77 women; age range, 36 to 76 years [mean, 61.0 years]). Of the 155 patients, 50 had chronic gastritis, 40 had gastric ulcer, 35 had duodenal ulcer, and 30 were diagnosed with intestinal-type gastric cancer. Informed consent was obtained from all patients, and the protocol was approved by the hospital ethics committee.

Histological evaluation. Three biopsy samples were taken from the greater curvature of the antrum. Two biopsy samples were used for histological examination, and one was used for *H. pylori* culture. One experienced pathologist blinded to the patient's clinical diagnosis and characteristics of the *H. pylori* strain examined the samples, stained with hematoxylin and eosin and modified Giemsa. Histological features were graded with the visual analog scale system according to the updated Sydney system (grades 0 to 3) (5). Each biopsy site was scored individually, and the median score was determined for the two biopsy sites.

***H. pylori* culture and preparation of *H. pylori* genomic DNA.** Bacterial isolates were subcultured at 37°C on brain heart infusion agar plates supplemented with 7% horse blood in a microaerobic atmosphere for 3 days. The organisms were identified as *H. pylori* by Gram staining, colony morphology, and positive oxidase, catalase, and urease reactions. The bacteria were harvested, and genomic DNA was extracted by using the QIAamp Tissue kit (Qiagen Inc., Santa Clarita, Calif.) according to the manufacturer's instructions.

PCR and DNA sequence analysis. Bacterial chromosomal DNA was added to 50-μl reaction mixtures containing 5 μl of 10× PCR buffer (500 mmol of KCl, 100 mmol of Tris-HCl [pH 8.8], and 15 mmol of $MgCl_2$ per liter; 1% Triton X-100; 200 mmol each of dATP, dCTP, dGTP, and dTTP [Promega Corporation, Madison, Wis.] and 200 nmol of each primer per liter; 1.0 U of *Taq* DNA polymerase [Perkin-Elmer Corporation]; and H_2O). The primers 5′ ACCCTA GTCGGTAATGGGTTA 3′ (CAG1) and 5′ GTAATTGTCTAGTTTCGC 3′ (CAG2) were used to amplify the 3′ region of the *cagA* gene. PCR was performed with a DNA Engine (MJ Research Inc., Watertown, Mass.) for 35 cycles, consisting of 1 min at 95°C, 1 min at 50°C, and 1 min at 72°C. The final cycle included a 7-min extension step to ensure full extension of the PCR product. PCR products were subcloned into the pCR 2.1 vector (Invitrogen, Carlsbad, Calif.) and sequenced by using an automated sequencer (University of Texas Houston Medical Center). The T3 and T7 primers, which anneal to vector regions flanking the cloning site, were used to sequence both strands of the cloned fragments. Sequence analysis was performed with the CLUSTAL W multiple-sequence alignment program, version 1.7 (18).

Immunoblotting assay for CagA protein. Whole-protein extracts from *H. pylori* isolates were obtained by resuspending the bacteria in 500 μl of Laemmli sample buffer, and proteins were denatured by incubation at 100°C for 10 min. The proteins were resolved by sodium dodecyl sulfate–8% polyacrylamide gel electrophoresis. The molecular weight determinations were performed with the Kaleidoscope prestained standards (Bio-Rad, Inc.). The relative molecular weights were determined by measuring distances from marker bands and the band corresponding to a control strain, CCUG17874, with a 128K CagA (3). The proteins were transferred onto Immobilon membranes (Millipore Corporation, Bedford, Mass.) and incubated with a 1:1,500 dilution of anti-recombinant CagA protein mouse polyclonal antibody (Oravax Inc., Cambridge, Mass.). After being washed, the filters were incubated with a 1:2,000 dilution of horseradish peroxidase-linked anti-mouse immunoglobulin antibody (Amersham Life Science Inc.). The proteins were then incubated with enhanced chemiluminescence detection reagents for 1 min, by using the ECL system (Amersham Life Science Inc.) and exposed to X-ray film.

Serum PG I and II assays. Serum samples were obtained from each patient on the day of the endoscopic procedure. Serum concentrations of pepsinogen (PG) I and PG II were measured by radioimmunoassay (Dainabot Co. Ltd., Tokyo, Japan), as instructed by the manufacturer.

Serum anti-CagA antibody assays. Microtiter wells were coated with 1 μg of orv220 antigen (Oravax Inc.), a 65,000-molecular-weight recombinant CagA protein purified from *Escherichia coli*, encoded by a fragment containing bp 1921 to 3648 of *cagA* (1), per well. Serum samples diluted 1/100 in 2.5% nonfat dried milk in phosphate-buffered saline-Tween were incubated at 37°C for 1 h. The secondary antibody, a goat anti-human immunoglobulin G-alkaline phosphatase conjugate, was then added at a dilution of 1:1,000 at 37°C for 1 h, followed by incubation with the alkaline phosphatase substrate at room temperature for 20 min, and the optical density was read at 405 nm. A standard curve of high-titer positive and control serum samples was included for each plate. Results were expressed in enzyme-linked immunosorbent assay (ELISA) units (on a scale of 0 to 100) determined from the standard curve. The cutoff was set at 5.0 ELISA units; it was calculated as the mean plus 3 standard deviations of the results obtained for 30 patients negative for *H. pylori* (27).

Data analysis. Fisher's exact test was used for analysis of categorical data. The Mann-Whitney U test was used for assessing differences between atrophy histological scores and *cagA* genotype. This test was also used for assessing differences between the PG I/II ratio and *cagA* genotype, differences between the molecular weight of CagA and the size of PCR product, and differences between the serum anti-CagA antibody units and the size of PCR product. Coefficients of correlation between the PG I/II ratio and atrophy score were calculated by the Spearman rank test. Data are presented as means ± standard deviations. A P value of <0.05 was accepted as statistically significant.

Nucleotide sequence accession numbers. The nucleotide sequences of JK25 (type A), JK252 (type B), JK269 (type C), and JK22 (type D) have been deposited in the GenBank database under accession no. AF043457, AF043458, AF043459, and AF043460, respectively.

RESULTS

PCR amplification products and primary structure of *cagA* genes. One hundred forty-five of 155 *H. pylori* strains (93.5%) examined by PCR with primers CAG1 and CAG2 yielded PCR products of similar sizes (*cagA* type A). The PCR products amplified from the remaining 10 strains had a larger size (Fig. 1). Sequencing of representative strains of the different sizes showed that their primary sequence and structural organization could be divided into four subtypes (A to D) (Fig. 2). Therefore, the combination of PCR and sequencing allowed us to distinguish four *cagA* types with different primary gene structures. Types A and C can be distinguished with PCR by their sizes, but types B and D have the same PCR product length and can be distinguished only by sequencing (Fig. 1 and 2).

To compare the sequences of the type A *cagA* genes, we randomly selected eight strains isolated from patients with gastritis, eight strains from patients with gastric ulcers, eight strains from patients with duodenal ulcers, and eight strains from patients with gastric cancer. Sequencing of DNA from *H. pylori* containing the type A gene showed that the amplified PCR products ranged from 642 to 651 bp. Sequence analysis revealed that type A strains were characterized by two repeats of 15-bp sequences (R1) and by a 42-bp region (R2) located between the two R1 repeats (Fig. 2). In the type A strains, immediately downstream of the R1-R2-R1 sequence we found a 147-bp segment (R3), followed by another R1 sequence (Fig.

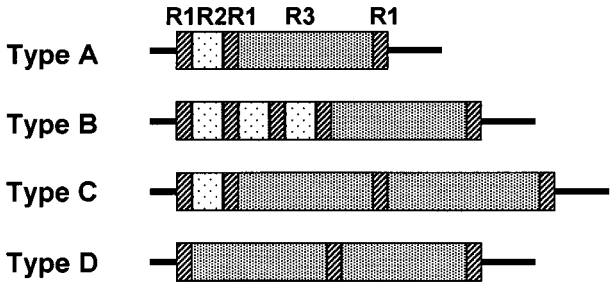

FIG. 2. Primary-structure variants of the 3′ region of the *cagA* gene in a Japanese population. The fragments are not represented on a proportional scale.

TABLE 1. Similarity analysis of the PCR products of type A *cagA* genes

Disease	Mean % identity (range) ($n = 8$)	
	Nucleotides	Amino acids
Chronic gastritis	97.8 (96.2–98.9)	96.4 (92.1–99.5)
Gastric ulcer	97.1 (95.8–98.3)	95.2 (92.1–98.1)
Duodenal ulcer	98.0 (97.2–98.8)	97.1 (93.5–98.6)
Gastric cancer	96.5 (95.1–97.2)	94.1 (92.2–97.7)

2). The consensus sequence for the type A *cagA* gene was determined by comparing the deduced amino acid sequences of the 32 type A strains sequenced. Comparison of the nucleotide and the deduced amino acid sequences of PCR amplification products from type A strains showed a high level of similarity among strains isolated from patients, independent of the *H. pylori*-related disease (Table 1).

Interestingly, the amino acid sequence of R3 regions differed from the corresponding reported sequences of *cagA* genes deposited in GenBank (GenBank accession no. L11714 [19], X70038 [11], and AB003397 [11]), with identities of only 45, 41, and 45%, respectively (Fig. 3B). More importantly, the sequence FPLKRHDKVDDLSKV in *H. pylori* strains from other geographic regions (3, 11, 19) was replaced by the sequence KIASAGKGVGGFSGA in the Japanese *H. pylori* strains.

Primary structure of type B, C, and D *cagA* variants. The type B *cagA* gene consisted of a 756-bp PCR product and was seen in two cases of chronic gastritis. A total of five repeats of sequence R1 was seen in the type B *cagA* gene (Fig. 2). Sequence analysis of the two type B strains showed that their nucleotide and deduced amino acid sequences had similarities of 99.5 and 100%, respectively. Comparison of the nucleotide sequences of type A and type B strains showed similarities of 95.2% for sequences R1 and R2 and 98.2% for sequences R3. The type C *cagA* gene consisted of 810- to 813-bp PCR products that contained an additional R3 sequence (Fig. 2). Among the type C *cagA* genes the nucleotide and deduced amino acid sequences between amino acid 880 and amino acid 974 showed similarities of 86.2 to 100% and 84.6 to 100%, respectively (Fig. 3A). In one patient with gastric ulcer a type D *cagA* gene was found. This type D gene resulted in a 756-bp PCR product and contained two R3 sequences but no R2 modules (Fig. 2).

In summary, the genetic diversity in the 3′ region of the *cagA* gene results either from amplification of the R1-R2 modules, as seen in *cagA* genes of type B, or from amplification of the R3 sequence module in strains of types C and D (Fig. 2).

Structural *cagA* variants result in larger-size CagA proteins. To investigate the relationship between variations in the sizes of the *cagA* PCR products and the relative molecular weights of CagA proteins, we performed immunoblotting assays with extracts from 50 *H. pylori* strains randomly selected from 145 strains with type A *cagA* and all 10 strains with larger-size PCR products (types B, C, and D). The median molecular weight of the CagA protein in strains which yielded a type A size PCR product was 134K (range, 129 to 138K). The molecular weights of CagA proteins were significantly greater in strains with larger-size PCR products than in strains with smaller-size PCR fragments ($P < 0.05$) (Table 2). All the type C strains from patients with gastric cancer had molecular weights between 141 and 142K (Fig. 4).

Association of *cagA* gene type with serum anti-CagA antibody. The ELISA titer of serum anti-CagA antibody in patients infected with type C strains was significantly greater than that in patients infected with type A strains (57.9 ± 12.0 [$n = 7$] versus 26.8 ± 10.4 [$n = 145$]; $P < 0.005$). To reduce possible bias caused by comparing results among patients with markedly different outcomes, we examined the serum anti-CagA antibody levels only in patients with gastric cancer. The mean score of serum anti-CagA antibody in patients infected with type C strains was significantly higher than that in patients infected with type A strains (57.7 ± 14.2 [$n = 6$] versus 20.1 ± 9.6 [$n = 24$]; $P < 0.005$) (Table 2).

Association of *cagA* gene type with specific clinical outcome and histological findings. Type C strains were disproportionally found among patients with gastric cancer versus noncancer patients ($P = 0.01$) (Table 3). In addition, patients infected with type C strains had severe atrophy (median score, 3) and low PG I/II ratios (mean, 1.6). As expected, the PG I/II ratio significantly correlated with the atrophy score when calculated for all patients ($n = 155$, $r = 0.803$; $P < 0.0001$). The patient infected with a type D strain had only mild atrophy. Patients with gastric cancer infected with type C strains ($n = 6$) had significantly higher atrophy scores and lower PG I/II ratios than those infected with type A strains ($n = 24$) (median atrophy score, 3 versus 2 [$P < 0.05$]; mean PG I/II ratio, 1.6 versus 4.2 [$P < 0.005$]) (Table 2). Patients infected with type B strains also had significantly higher atrophy scores and lower PG I/II ratios than those infected with type A strains (median atrophy score, 3 versus 2 [$P < 0.05$]; mean PG I/II ratio, 1.2 versus 3.4 [$P < 0.005$]).

DISCUSSION

The *cagA* gene is a putative *H. pylori* virulence factor of unknown function. The primary sequence of the *cagA* gene contains repeat sequences in the 3′ region (3, 11, 19). This study evaluated the structure of this region of the *cagA* gene in a geographic area where symptomatic *H. pylori*-related disease is common and also asked whether there was a biological significance to the variability in the structural organization of the 3′ region of *cagA*. In Japan at least 90% of the *H. pylori* isolates contain the *cagA* gene (10, 16, 25, 26).

PCR and sequencing of the PCR products led to the identification of four types of the *cagA* gene (types A to D) that differed in the structural organization of their primary sequences, as a result of variation in the numbers of different repeat regions. We elected to designate the repeats of Japanese strains as R1, R2, and R3. Interestingly, the sequence of R3 regions of the *cagA* gene in Japan differed markedly from the reported sequences of *cagA* genes in Western isolates (3, 11, 19), with identities of only 41 to 45%. The most frequent type of *cagA* 3′ region (type A) ranged in PCR product size from 642 to 651 bp. The second most frequent type of *cagA* structure in Japan was type C, which contained two copies of the R3 region, with each copy being flanked by R1 regions. Western blot analysis of CagA proteins confirmed that the repeat regions resulted in proteins of increased size. For example, the type C *cagA* genes which contain two R3 regions resulted in CagA proteins of larger size (141 to 142K), and the type A strains with only one R3 repeat ranged in size from 129 to 138K.

We examined the possibility that the different structural subtypes of the *cagA* gene may be preferentially associated with specific *H. pylori*-related gastric diseases and found that 86% of type C variants were obtained from patients with gastric cancer; one type C strain was present in a patient with gastric ulcer, a disease that has been associated with an increased risk of gastric cancer development (17).

Many studies have supported the notion that gastric cancer is a multifactorial disease, making it essential to take into

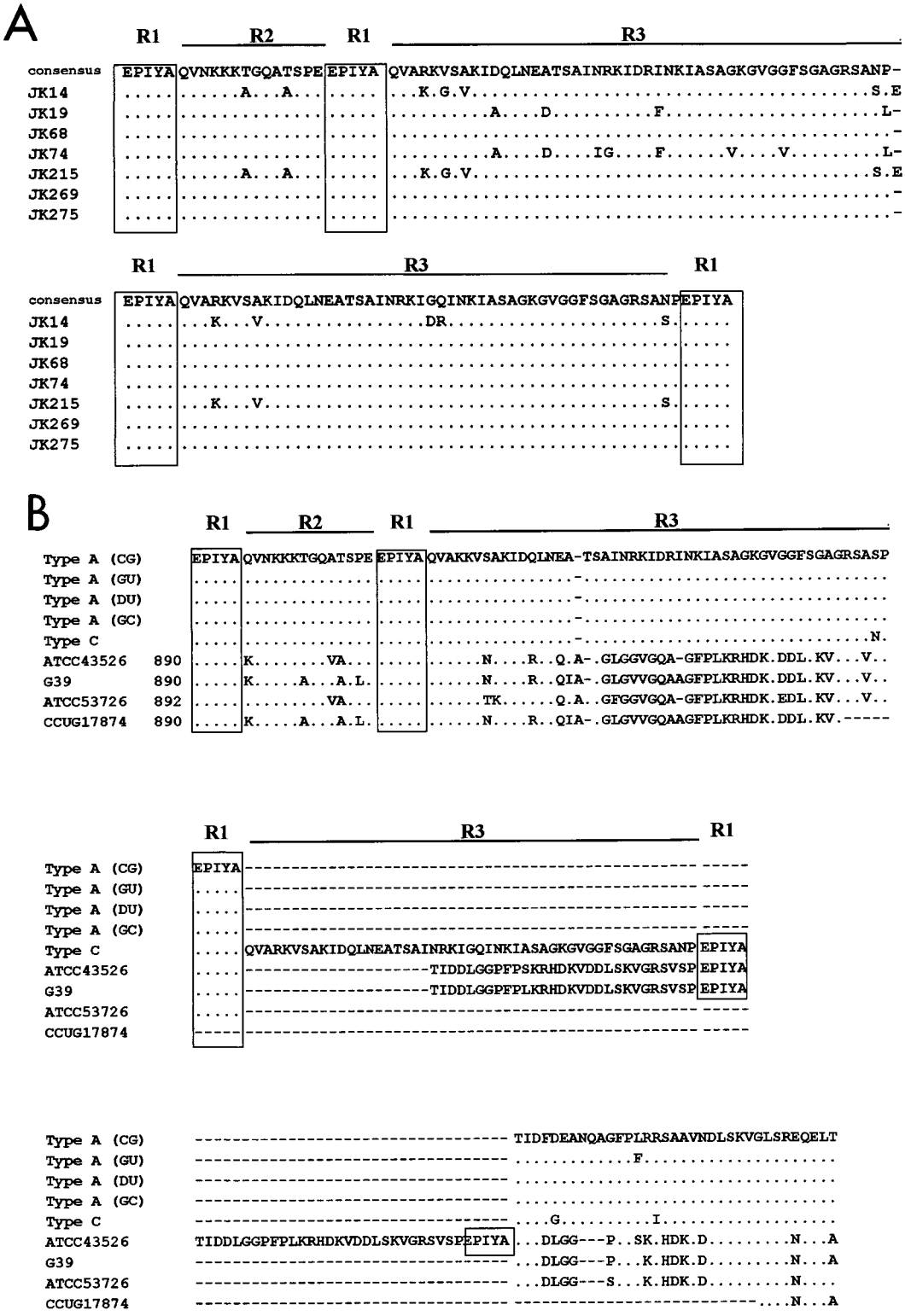

FIG. 3. Sequence analyses of type A and type C *cagA* genes. (A) Alignment of the deduced amino acid sequences of type C strains, in the region corresponding to amino acid residues 892 to 969 of the *H. pylori* ATCC 53726 CagA product (GenBank accession no. L11714). Strain JK14 was from a patient with gastric ulcer, and the remaining six strains were from patients with gastric cancer. (B) Comparison of the deduced amino acid sequences of the R1, R2, and R3 regions of type A and type C strains with the same region of the gene product of a reference strain. The reference strains ATCC 43526, G39, ATCC 53726, and CCUG17874 (GenBank accession no. AB003397, X70038, L11714, and X70039, respectively) were used for sequence comparison. Consensus sequences among the *cagA* genes from *H. pylori* isolates from patients with chronic gastritis (CG), gastric ulcer (GU), duodenal ulcer (DU), and gastric cancer (GC) were separately determined among type A strains.

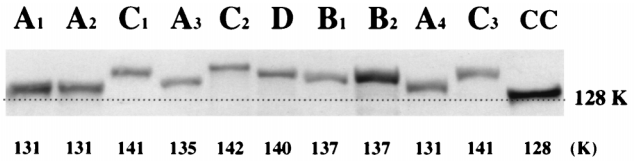

FIG. 4. Western blot analysis of CagA proteins. A_1 through A_4, B_1 and B_2, C_1 through C_3, and D represent examples of strains with 3′ region structures that characterize *cagA* genes of types A to D. The molecular weights of proteins were determined in comparison to molecular weight standards run on the same gel (Kaleidoscope prestained standards) and by comparison with the size of CagA from the reference strain CCUG17874 (128K) (lane CC). The molecular weights of the CagA proteins are indicated at the bottom.

TABLE 3. Relationship between the structure of the *cagA* gene 3′ region and *H. pylori*-related diseases

Strain type	Chronic gastritis (n = 50)	Gastric ulcer (n = 40)	Duodenal ulcer (n = 35)	Gastric cancer (n = 30)
A	48	38	35	24
B	2	0	0	0
C	0	1	0	6[a]
D	0	1	0	0

[a] The percentage of type C strains was significantly higher in the gastric cancer group than in other groups (chronic gastritis, $P < 0.005$; gastric ulcer, $P < 0.05$; duodenal ulcer, $P < 0.01$).

account bacterial virulence factors that might be associated with increased pathogenicity of specific *H. pylori* strains. Our study differs from prior studies of the association of CagA and gastric cancer, as it is not a seroepidemiological study but, instead, used genetic analyses to investigate a possible relationship between the *cagA* gene substructure, the precursor lesion of gastric cancer (atrophy), and gastric cancer. Of particular interest is the relationship between the degree of atrophic gastritis and the subtype of *cagA* structure, with both type B and type C infections being associated with severe atrophic gastritis.

It has been suggested that the presence of repeat regions in the 3′ region of the *cagA* gene may result in proteins with different immunogenicities (3). It is unlikely that the presence of these repeats is useful for generating antigenic diversity, because within the same geographic population, the primary gene sequence of these regions is significantly conserved. The presence of multiple repeats may also generate immunodominant nonprotective epitopes (3).

The orv220 antigen used in ELISAs does not contain the R1, R2, and R3 repeat regions. Nevertheless, we found that the mean ELISA titer of serum anti-CagA antibody in patients infected with type C strains was significantly higher than that in patients infected with type A *cagA* strains, possibly indicating a stronger, more persistent immune response. The role of inflammatory mediators in gastric carcinogenesis has been suggested in several studies, and our finding of a higher titer of serum anti-CagA antibodies in patients harboring type C strains is in agreement with this hypothesis. The fact that patients with gastric cancer and type C strains had severe atrophy also supports this view, because higher levels of inflammation in the gastric mucosa in the early stages of natural progression of the infection might have been an important factor in the development of severe atrophy.

In conclusion, studies of the primary gene structure of the 3′ region of the *cagA* gene of *H. pylori* isolates in Japan showed that this region of *cagA* differs markedly from the primary sequence of *cagA* genes reported for Western isolates (3, 11, 19). In particular, the sequence FPLKRHDKVDDLSKV in *H. pylori* strains from other geographic regions was replaced by the sequence KIASAGKGVGGFSGA in the Japanese *H. pylori* strains. These differences in primary structure are detectable by PCR analysis, can be easily used to identify the geographic region of origin of *H. pylori* isolates, and can therefore be useful for epidemiological studies. In addition, our preliminary findings suggest that a structural subtype of the 3′ region (type C) of the *cagA* gene results in CagA proteins with higher molecular weights and is associated with gastric atrophy and carcinoma. These data suggest that *cagA* variants may provide new markers for other factors involved in gastric carcinogenesis or may be associated with higher levels of immune response, possibly influencing the outcome of *H. pylori* infection.

TABLE 2. PCR amplification products and CagA proteins from Japanese patients with gastric carcinoma

Patient[a]	Age (yr)	Sex[b]	Cancer stage	Atrophy score (median)	PG I/II ratio	Anti-CagA score[c]	Strain type	PCR product (bp)	CagA protein (K)[d]
JK 19	46	M	Early	2.5	1.4	52.5	C	810	141
JK 68	76	F	Early	3	1.0	23.9	C	810	141
JK 74	73	F	Advanced	3	2.3	100	C	810	141
JK 215	62	M	Early	3	1.8	46.5	C	810	141
JK 269	60	M	Early	3	1.0	23.1	C	810	142
JK 275	55	F	Advanced	3	1.8	100	C	810	142
JK 260	75	M	Early	1	3.0	15.5	A	648	129
JK 273	59	M	Early	0.5	5.9	7.2	A	648	129
JK 256	68	F	Early	1	2.7	7.4	A	648	135
JK 69	76	F	Early	3	1.3	19.7	A	648	135
JK 83	71	M	Early	2	1.6	8.1	A	651	135
JK 34	28	M	Early	1	4.1	22.6	A	648	135
JK 42	76	M	Early	2	1.5	36.7	A	642	135
JK 217	64	F	Early	2	2.1	9.5	A	642	137
JK 96	59	M	Early	1	5.9	8.2	A	648	136
JK 55	68	M	Early	1	2.7	96.1	A	651	129

[a] Data for strains from the remaining patients is not shown because sequence analysis or Western blotting was not performed; however, atrophy scores, PG I/II ratios, anti-CagA scores, and PCR product sizes were available for analysis (see Results).
[b] M, male; F, female.
[c] Cutoff, 5.0.
[d] Determined by immunoblotting.

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